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Original article

Quorum sensing suggesting activities of algicidal bacteria that kill red tide raphidophyte *Heterosigma akashiwo* and *Chattonella antiqua*

Isamu Kodama [1,*], Nobuharu Inaba [2] and Ichiro Imai [1]

[1] *Graduate School of Fisheries Science, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido, 041-8611, Japan*
[2] *National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency, Kanazawa-ku, Yokohama, Kanagawa, 236-8648, Japan*
* Correspondence: k-damakan@fish.hokudai.ac.jp; Tel.: +81 138 40 5543

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Introduction

Harmful algal blooms (HABs) have occurred with increasing frequency in the coastal environments of the world [1,2] and huge fishery damages have recurrently been given to cultured fish and bivalves [3]. There is consequently an urgent need for the development of feasible tools for reducing the negative impacts of HABs. Algicidal bacteria (AB) and growth–inhibiting bacteria (GIB) against HAB species could potentially serve as environment friendly tools in reducing the impacts of HABs [4]. Large numbers of AB and GIB have been detected from the biofilms of the surface of seaweeds and seagrasses, and also distributed in the water of seaweed and seagrass beds [5,6]. In order to use these AB and GIB as a HAB control method, it is basically important to elucidate the algicidal and growth–inhibiting mechanism. Previous researches suggested that quorum sensing (QS), communication mechanism of bacteria, is involved in algicidal and growth–inhibiting mechanism by bacteria [7-10]. In the present paper, we describe algicidal and growth–inhibiting activities of AB and GIB isolated from seagrass beds, and suggest an involvement of QS in the algicidal mechanism.

Materials and methods

Bacterial strains of AB and used in this study were isolated from the biofilms on surface of the seagrass *Zostera marina* collected in Puget Sound, WA, USA [11] and the coast of Hinase Islands, Okayama Prefecture, Japan. Bacterial strains preserved at -80˚C were restored and cultured in $ST10^{-1}$ liquid or agar medium [12] at 20˚C. Two species of harmful microalgae belonging to Raphidophyceae (*Heterosigma akashiwo* 893 and *Chattonella antiqua* NIES–1) were used as prey organisms. All axenic algal cultures were maintained in the modified SWM–3 culture medium [13,14], at 20˚C for *H. akashiwo* and 25˚C for *C. antiqua* and a light intensity of about 50–100 µmol photons m^{-1} s^{-1} with a 14 h light: 10 h dark photo–cycle. Algal pre–cultures grown to the early stationary phase were diluted with the same medium (final cell densities were about 10^3 cells mL^{-1} for *C. antiqua* and about 10^4 cells mL^{-1} for *H. akashiwo*), and 5 mL aliquots were pipetted into test tubes. Each strain of AB and GIB was grown for 3–5 days in the liquid $ST10^{-1}$ medium and cell densities reached about 10^8 cells mL^{-1}. The bacterial cultures were then diluted with the SWM–3 medium, and 50 µL aliquots were inoculating at final concentrations of about 10^3 cells mL^{-1} to the tubes including algae (liquid culture experiment). Further, a small amount of the cake of bacterial strains colonized on agar $ST10^{-1}$ medium was added to the test tubes using autoclaved toothpicks (colony cake experiment). Control was no additional of bacteria to algal cultures. Triplicate test tubes were used for each co–culture experiment. Inoculated samples were incubated for 14–15 days under the same conditions of maintenance of algae as described above. The growth as survival (or death) of phytoplankton in tubes was monitored by in vivo fluorescence using a fluorometer [15,16]. Monitoring intervals were every day for the liquid culture experiment, and every two days from the third day for the colony cake additional experiment.

Results and discussion

Figure 1 shows the results of co–culture experiment of *H. akashiwo* and the bacterium strain 3 (*Pseudoalteromonas* sp., originally isolated against *H. akashiwo*). Figure 2 also shows the results of *C. antiqua* and two bacterial strains (strain 138 and 153, both *Phaeobacter* spp., originally isolated against *C. antiqua*). The fluorescence values of the control of *H. akashiwo* steadily increased from 4.4 to 124 from the start of experiment (Fig. 1), and the control of *C. antiqua* grew from 3.4 to 44.3 on the 13 and gradually decreased thereafter (Fig. 2). As a result of co–culture

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experiment in *H. akashiwo*, a highly effective algicidal activity was observed when bacterial cells were added as aggregation to the algal culture (i.e. colony cake experiment) than floating cell suspension (liquid culture experiment). As shown in Fig. 1, the growths of

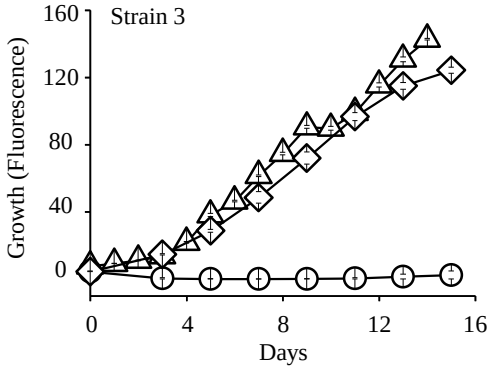

Fig. 1. Effects of the algicidal bacterium strain 3 against *Heterosigma akashiwo* (◇: growth of *H. akashiwo* with no addition of bacteria; △: growth with bacterial inoculation as cell suspension (liquid culture); ○: growth with inoculation of colony cake of bacteria.

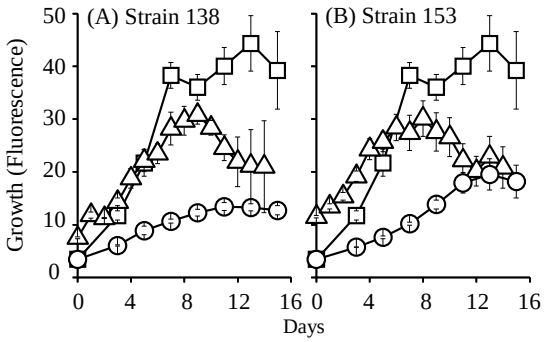

Fig. 2. Effects of the growth-inhibition bacteria strains 138 (A) and 153 (B) against *Chattonella antiqua* (□: growth of C. antiqua with no addition of bacteria; △: growth with bacterial inoculation as cell suspension (liquid culture); ○: growth with inoculation of colony cake of bacteria.

H. akashiwo were similar in the control and liquid culture experiments. The fluorescence values in colony cake addition experiment for *H. akashiwo* stayed 0.01 to 4.4 throughout the experiment and entire death was observed. The bacterial strains 138 and 153 showed growth–inhibiting activities against *C. antiqua* when bacterial cells were added as a small colony cake. However, *C. antiqua* in liquid culture experiment showed similar growth as control until 9, and then decreased. It was considered that bacterial density was locally very high in colony cake addition experiment, and algicidal activity was induced by quorum sensing [17]. In liquid culture experiments, it is considered the bacterial densities did not reach the level of quorum sensing induction until day 9, and reached thereafter. QS is reported in the regulation of algicidal activity against marine harmful algal species and toxic freshwater cyanobacteria [7-10]. On the other hand, some previous studies reported strong algicidal bacteria which can kill all cells of *H. akashiwo* and *C. antiqua* in the bottle when only a few cells were inoculated into the culture in the bottle [15,16].

This study suggested that QS is involved in the algicidal mechanisms of AB and GIB. In order to use AB and GIB for HABs control, it is important to elucidate the role of QS in algicidal and growth–inhibiting mechanisms, and to find the methods inducing QS in the field of the sea.

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